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DOMPATENT VON KREISLER KOELN

NR. 2310 S. 38/61

Express Mail No. EV529815955US

10/588570**IAP11 Rec'd PCT/PTO 03 AUG 2006**Sequence Listing

<110> Rheinische Friedrich-Wilhelms Universität Bonn

<120> Mutated DNA polymerases with increased mispairing discrimination

<130> 050188wo JH/PCH

<140>

<141>

<150> DE 102004005885.7

<151> 2004-02-05

<160> 29

<170> PatentIn Ver. 2.1

<210> 1

<211> 2787

<212> DNA

<213> artificial sequence

<220>

<223> Description of artificial sequence: *E. coli*

Wild type Klenow fragment of DNA polymerase 1

<400> 1

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ggtgtcctca acatgctcgc cagtctgata atgcaatata aaccgacgca tgcagcgggtg 180
gtctttgacg ccaagggaaa aacctttcgt gatgaactgt ttgaacatta caaatcacat 240
cgcccgccaa tgccggacga tctgcgtgca caaatcgaa ccttgcaacg gatgggtaaa 300
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cttcgtctgg ctgagctgga aaagaaagcg catgaaattg caggtgagga atttaacctt 1740
tcttcacca agcagttaca aaccattctc tttgaaaaac agggcattaa accgctgaag 1800
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```

aaaacgccgg gtggcgccgc gtcaacgtcg gaagaggtac tggagaact ggcgctggac 1860
tatccgttgc caaaagtgat tctggagtat cgtggtctgg cgaagctgaa atcgacctac 1920
accgacaagc tgccgctgat gatcaaccgc aaaaccgggc gtgtgcatac ctcttatcac 1980
caggcagtaa ctgcaacggg acgtttatcg tcaaccgatc ctaacctgca aaacattccg 2040
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<210> 2

<211> 928

<212> PRT

<213> artificial sequence

<220>

<223> Description of artificial sequence: *E. coli*

Klenow fragment of DNA polymerase 1

<400> 2

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Met Val Gln Ile Pro Gln Asn Pro Leu Ile Leu Val Asp Gly Ser Ser
 1             5             10             15

```

```

Tyr Leu Tyr Arg Ala Tyr His Ala Phe Pro Pro Leu Thr Asn Ser Ala
      20             25             30

```

```

Gly Glu Pro Thr Gly Ala Met Tyr Gly Val Leu Asn Met Leu Arg Ser
      35             40             45

```

```

Leu Ile Met Gln Tyr Lys Pro Thr His Ala Ala Val Val Phe Asp Ala
      50             55             60

```

```

Lys Gly Lys Thr Phe Arg Asp Glu Leu Phe Glu His Tyr Lys Ser His
      65             70             75             80

```

```

Arg Pro Pro Met Pro Asp Asp Leu Arg Ala Gln Ile Glu Pro Leu His
      85             90             95

```

```

Ala Met Val Lys Ala Met Gly Leu Pro Leu Leu Ala Val Ser Gly Val
      100            105            110

```

```

Glu Ala Asp Asp Val Ile Gly Thr Leu Ala Arg Glu Ala Glu Lys Ala
      115            120            125

```

```

Gly Arg Pro Val Leu Ile Ser Thr Gly Asp Lys Asp Met Ala Gln Leu
      130            135            140

```

```

Val Thr Pro Asn Ile Thr Leu Ile Asn Thr Met Thr Asn Thr Ile Leu
      145            150            155            160

```

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- 3 -

Gly Pro Glu Glu Val Val Asn Lys Tyr Gly Val Pro Pro Glu Leu Ile
 165 170 175
 Ile Asp Phe Leu Ala Leu Met Gly Asp Ser Ser Asp Asn Ile Pro Gly
 180 185 190
 Val Pro Gly Val Gly Glu Lys Thr Ala Gln Ala Leu Leu Gln Gly Leu
 195 200 205
 Gly Gly Leu Asp Thr Leu Tyr Ala Glu Pro Glu Lys Ile Ala Gly Leu
 210 215 220
 Ser Phe Arg Gly Ala Lys Thr Met Ala Ala Lys Leu Glu Gln Asn Lys
 225 230 235 240
 Glu Val Ala Tyr Leu Ser Tyr Gln Leu Ala Thr Ile Lys Thr Asp Val
 245 250 255
 Glu Leu Glu Leu Thr Cys Glu Gln Leu Glu Val Gln Gln Pro Ala Ala
 260 265 270
 Glu Glu Leu Leu Gly Leu Phe Lys Lys Tyr Glu Phe Lys Arg Trp Thr
 275 280 285
 Ala Asp Val Glu Ala Gly Lys Trp Leu Gln Ala Lys Gly Ala Lys Pro
 290 295 300
 Ala Ala Lys Pro Gln Glu Thr Ser Val Ala Asp Glu Ala Pro Glu Val
 305 310 315 320
 Thr Ala Thr Val Ile Ser Tyr Asp Asn Tyr Val Thr Ile Leu Asp Glu
 325 330 335
 Glu Thr Leu Lys Ala Trp Ile Ala Lys Leu Glu Lys Ala Pro Val Phe
 340 345 350
 Ala Phe Asp Thr Glu Thr Asp Ser Leu Asp Asn Ile Ser Ala Asn Leu
 355 360 365
 Val Gly Leu Ser Phe Ala Ile Glu Pro Gly Val Ala Ala Tyr Ile Pro
 370 375 380
 Val Ala His Asp Tyr Leu Asp Ala Pro Asp Gln Ile Ser Arg Glu Arg
 385 390 395 400
 Ala Leu Glu Leu Leu Lys Pro Leu Leu Glu Asp Glu Lys Ala Leu Lys
 405 410 415
 Val Gly Gln Asn Leu Lys Tyr Asp Arg Gly Ile Leu Ala Asn Tyr Gly
 420 425 430
 Ile Glu Leu Arg Gly Ile Ala Phe Asp Thr Met Leu Glu Ser Tyr Ile
 435 440 445
 Leu Asn Ser Val Ala Gly Arg His Asp Met Asp Ser Leu Ala Glu Arg
 450 455 460
 Trp Leu Lys His Lys Thr Ile Thr Phe Glu Glu Ile Ala Gly Lys Gly
 465 470 475 480

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Lys Asn Gln Leu Thr Phe Asn Gln Ile Ala Leu Glu Glu Ala Gly Arg
 485 490 495
 Tyr Ala Ala Glu Asp Ala Asp Val Thr Leu Gln Leu His Leu Lys Met
 500 505 510
 Trp Pro Asp Leu Gln Lys His Lys Gly Pro Leu Asn Val Phe Glu Asn
 515 520 525
 Ile Glu Met Pro Leu Val Pro Val Leu Ser Arg Ile Glu Arg Asn Gly
 530 535 540
 Val Lys Ile Asp Pro Lys Val Leu His Asn His Ser Glu Glu Leu Thr
 545 550 555 560
 Leu Arg Leu Ala Glu Leu Glu Lys Lys Ala His Glu Ile Ala Gly Glu
 565 570 575
 Glu Phe Asn Leu Ser Ser Thr Lys Gln Leu Gln Thr Ile Leu Phe Glu
 580 585 590
 Lys Gln Gly Ile Lys Pro Leu Lys Lys Thr Pro Gly Gly Ala Pro Ser
 595 600 605
 Thr Ser Glu Glu Val Leu Glu Glu Leu Ala Leu Asp Tyr Pro Leu Pro
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 Lys Val Ile Leu Glu Tyr Arg Gly Leu Ala Lys Leu Lys Ser Thr Tyr
 625 630 635 640
 Thr Asp Lys Leu Pro Leu Met Ile Asn Pro Lys Thr Gly Arg Val His
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 Thr Ser Tyr His Gln Ala Val Thr Ala Thr Gly Arg Leu Ser Ser Thr
 660 665 670
 Asp Pro Asn Leu Gln Asn Ile Pro Val Arg Asn Glu Glu Gly Arg Arg
 675 680 685
 Ile Arg Gln Ala Phe Ile Ala Pro Glu Asp Tyr Val Ile Val Ser Ala
 690 695 700
 Asp Tyr Ser Gln Ile Glu Leu Arg Ile Met Ala His Leu Ser Arg Asp
 705 710 715 720
 Lys Gly Leu Leu Thr Ala Phe Ala Glu Gly Lys Asp Ile His Arg Ala
 725 730 735
 Thr Ala Ala Glu Val Phe Gly Leu Pro Leu Glu Thr Val Thr Ser Glu
 740 745 750
 Gln Arg Arg Ser Ala Lys Ala Ile Asn Phe Gly Leu Ile Tyr Gly Met
 755 760 765
 Ser Ala Phe Gly Leu Ala Arg Gln Leu Asn Ile Pro Arg Lys Glu Ala
 770 775 780

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Gln Lys Tyr Met Asp Leu Tyr Phe Glu Arg Tyr Pro Gly Val Leu Glu
 785 790 795 800

Tyr Met Glu Arg Thr Arg Ala Gln Ala Lys Glu Gln Gly Tyr Val Glu
 805 810 815

Thr Leu Asp Gly Arg Arg Leu Tyr Leu Pro Asp Ile Lys Ser Ser Asn
 820 825 830

Gly Ala Arg Arg Ala Ala Ala Glu Arg Ala Ala Ile Asn Ala Pro Met
 835 840 845

Gln Gly Thr Ala Ala Asp Ile Ile Lys Arg Ala Met Ile Ala Val Asp
 850 855 860

Ala Trp Leu Gln Ala Glu Gln Pro Arg Val Arg Met Ile Met Gln Val
 865 870 875 880

His Asp Glu Leu Val Phe Glu Val His Lys Asp Asp Val Asp Ala Val
 885 890 895

Ala Lys Gln Ile His Gln Leu Met Glu Asn Cys Thr Arg Leu Asp Val
 900 905 910

Pro Leu Leu Val Glu Val Gly Ser Gly Glu Asn Trp Asp Gln Ala His
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<210> 3

<211> 2499

<212> DNA

<213> artificial sequence

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<223> Description of artificial sequence: Wild type Taq polymerase

<400> 3

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 tacaaggcgg gccgggcccc cagcccgagg gactttcccc ggcaactcgc cctcatcaag 300
 gagctggtgg acctcctggg gctggcgccg ctcgagggtc cgggctacga ggcggacgac 360
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 gccgacaaag acctttacca gctcctttcc gaccgcatcc acgtcctcca ccccgagggg 480
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- 6 -

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<210> 4

<211> 832

<212> PRT

<213> Artificial sequence

<220>

<223> Description of artificial sequence: Wild type Taq polymerase

<400> 4

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Met Arg Gly Met Leu Pro Leu Phe Glu Pro Lys Gly Arg Val Leu Leu
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```

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Val Asp Gly His His Leu Ala Tyr Arg Thr Phe His Ala Leu Lys Gly
      20             25             30

```

```

Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe Ala
      35             40             45

```

```

Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Asp Ala Val Ile Val
      50             55             60

```

```

Val Phe Asp Ala Lys Ala Pro Ser Phe Arg His Glu Ala Tyr Gly Gly
      65             70             75             80

```

```

Tyr Lys Ala Gly Arg Ala Pro Thr Pro Glu Asp Phe Pro Arg Gln Leu
      85             90             95

```

```

Ala Leu Ile Lys Glu Leu Val Asp Leu Leu Gly Leu Ala Arg Leu Glu
      100            105            110

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Val Pro Gly Tyr Glu Ala Asp Asp Val Leu Ala Ser Leu Ala Lys Lys
      115            120            125

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Ala Glu Lys Glu Gly Tyr Glu Val Arg Ile Leu Thr Ala Asp Lys Asp
 130 135 140
 Leu Tyr Gln Leu Leu Ser Asp Arg Ile His Val Leu His Pro Glu Gly
 145 150 155 160
 Tyr Leu Ile Thr Pro Ala Trp Leu Trp Glu Lys Tyr Gly Leu Arg Pro
 165 170 175
 Asp Gln Trp Ala Asp Tyr Arg Ala Leu Thr Gly Asp Glu Ser Asp Asn
 180 185 190
 Leu Pro Gly Val Lys Gly Ile Gly Glu Lys Thr Ala Arg Lys Leu Leu
 195 200 205
 Glu Glu Trp Gly Ser Leu Glu Ala Leu Leu Lys Asn Leu Asp Arg Leu
 210 215 220
 Lys Pro Ala Ile Arg Glu Lys Ile Leu Ala His Met Asp Asp Leu Lys
 225 230 235 240
 Leu Ser Trp Asp Leu Ala Lys Val Arg Thr Asp Leu Pro Leu Glu Val
 245 250 255
 Asp Phe Ala Lys Arg Arg Glu Pro Asp Arg Glu Arg Leu Arg Ala Phe
 260 265 270
 Leu Glu Arg Leu Glu Phe Gly Ser Leu Leu His Glu Phe Gly Leu Leu
 275 280 285
 Glu Ser Pro Lys Ala Leu Glu Glu Ala Pro Trp Pro Pro Pro Glu Gly
 290 295 300
 Ala Phe Val Gly Phe Val Leu Ser Arg Lys Glu Pro Met Trp Ala Asp
 305 310 315 320
 Leu Leu Ala Leu Ala Ala Arg Gly Gly Arg Val His Arg Ala Pro
 325 330 335
 Glu Pro Tyr Lys Ala Leu Arg Asp Leu Lys Glu Ala Arg Gly Leu Leu
 340 345 350
 Ala Lys Asp Leu Ser Val Leu Ala Leu Arg Glu Gly Leu Gly Leu Pro
 355 360 365
 Pro Gly Asp Asp Pro Met Leu Leu Ala Tyr Leu Leu Asp Pro Ser Asn
 370 375 380
 Thr Thr Pro Glu Gly Val Ala Arg Arg Tyr Gly Gly Glu Trp Thr Glu
 385 390 395 400
 Glu Ala Gly Glu Arg Ala Ala Leu Ser Glu Arg Leu Phe Ala Asn Leu
 405 410 415
 Trp Gly Arg Leu Glu Gly Glu Glu Arg Leu Leu Trp Leu Tyr Arg Glu
 420 425 430
 Val Glu Arg Pro Leu Ser Ala Val Leu Ala His Met Glu Ala Thr Gly
 435 440 445

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Val Arg Leu Asp Val Ala Tyr Leu Arg Ala Leu Ser Leu Glu Val Ala
 450 455 460
 Glu Glu Ile Ala Arg Leu Glu Ala Glu Val Phe Arg Leu Ala Gly His
 465 470 475 480
 Pro Phe Asn Leu Asn Ser Arg Asp Gln Leu Glu Arg Val Leu Phe Asp
 485 490 495
 Glu Leu Gly Leu Pro Ala Ile Gly Lys Thr Glu Lys Thr Gly Lys Arg
 500 505 510
 Ser Thr Ser Ala Ala Val Leu Glu Ala Leu Arg Glu Ala His Pro Ile
 515 520 525
 Val Glu Lys Ile Leu Gln Tyr Arg Glu Leu Thr Lys Leu Lys Ser Thr
 530 535 540
 Tyr Ile Asp Pro Leu Pro Asp Leu Ile His Pro Arg Thr Gly Arg Leu
 545 550 555 560
 His Thr Arg Phe Asn Gln Thr Ala Thr Ala Thr Gly Arg Leu Ser Ser
 565 570 575
 Ser Asp Pro Asn Leu Gln Asn Ile Pro Val Arg Thr Pro Leu Gly Gln
 580 585 590
 Arg Ile Arg Arg Ala Phe Ile Ala Glu Glu Gly Trp Leu Leu Val Ala
 595 600 605
 Leu Asp Tyr Ser Gln Ile Glu Leu Arg Val Leu Ala His Leu Ser Gly
 610 615 620
 Asp Glu Asn Leu Ile Arg Val Phe Gln Glu Gly Arg Asp Ile His Thr
 625 630 635 640
 Glu Thr Ala Ser Trp Met Phe Gly Val Pro Arg Glu Ala Val Asp Pro
 645 650 655
 Leu Met Arg Arg Ala Ala Lys Thr Ile Asn Phe Gly Val Leu Tyr Gly
 660 665 670
 Met Ser Ala His Arg Leu Ser Gln Glu Leu Ala Ile Pro Tyr Glu Glu
 675 680 685
 Ala Gln Ala Phe Ile Glu Arg Tyr Phe Gln Ser Phe Pro Lys Val Arg
 690 695 700
 Ala Trp Ile Glu Lys Thr Leu Glu Glu Gly Arg Arg Arg Gly Tyr Val
 705 710 715 720
 Glu Thr Leu Phe Gly Arg Arg Arg Tyr Val Pro Asp Leu Glu Ala Arg
 725 730 735
 Val Lys Ser Val Arg Glu Ala Ala Glu Arg Met Ala Phe Asn Met Pro
 740 745 750

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Val Gln Gly Thr Ala Ala Asp Leu Met Lys Leu Ala Met Val Lys Leu
 755 760 765

Phe Pro Arg Leu Glu Glu Met Gly Ala Arg Met Leu Leu Gln Val His
 770 775 780

Asp Glu Leu Val Leu Glu Ala Pro Lys Glu Arg Ala Glu Ala Val Ala
 785 790 795 800

Arg Leu Ala Lys Glu Val Met Glu Gly Val Tyr Pro Leu Ala Val Pro
 805 810 815

Leu Glu Val Glu Val Gly Ile Gly Glu Asp Trp Leu Ser Ala Lys Glu
 820 825 830

<210> 5

<211> 39

<212> DNA

<213> Artificial sequence

<220>

<223> Description of artificial sequence: Primer

<400> 5

gtacgtatga tcatgnnnnn nnnngatgaa ctggtattt

39

<210> 6

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> Description of artificial sequence:
 Downstream primer

<400> 6

gctaattaag cttggctgca ggc

23

<210> 7

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Description of artificial sequence:
 Antisense primer

<400> 7

tacatggacc ttacttcga acgc

24

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<210> 8
<211> 20
<212> DNA
<213> Artificial sequence

<220>
<223> Description of artificial sequence: Primer
FVL20TH

<400> 8
acaaaatacc tgtattcctt 20

<210> 9
<211> 90
<212> DNA
<213> Artificial sequence

<220>
<223> Description of artificial sequence: Template
TFVL90A

<400> 9
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tccctggaca ggcaaggaat acaggtattt 90

<210> 10
<211> 90
<212> DNA
<213> Artificial sequence

<220>
<223> Description of artificial sequence: Template
TFVL90G

<400> 10
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tccctggaca ggcgaggaat acaggtattt 90

<210> 11
<211> 20
<212> DNA
<213> Artificial sequence

<220>
<223> Description of artificial sequence: Primer for the
detection of the SNP in human genomic factor V
Leiden DNA sequence

<400> 11
acaaaatacc tgtattcctn 20

<210> 12
<211> 35
<212> DNA
<213> Artificial sequence

- 11 -

<220>

<223> Description of artificial sequence: Template of
human genomic factor V Leiden DNA sequence;
n = g, wild type template; n = a, mutant template

<400> 12

gatccctgga caggcnagga atacaggtat tttgt

35

<210> 13

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Description of artificial sequence: Primer for the
detection of human somatic BRAF T1796A mutation

<400> 13

gaccactcc atcgagattt ct

22

<210> 14

<211> 35

<212> DNA

<213> Artificial sequence

<220>

<223> Description of artificial sequence: Wild type
template of BRAF gene; w = t, wild type template;
w = a, mutant template

<400> 14

ggcttagcta cagwgaaatc tcgatggagt gggtc

35

<210> 15

<211> 25

<212> DNA

<213> Artificial sequence

<220>

<223> Description of artificial sequence: Primer for the
detection of human dihydropyrimidine dehydrogenase
(DPyD) mutation G735A

<400> 15

gttttagatg ttaaatacaca cttat

25

<210> 16

<211> 38

<212> DNA

<213> Artificial sequence

<220>

- 12 -

<223> Description of artificial sequence: Template of
human DPyD; r = g, wild type template; r = a,
mutant template

<400> 16

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38

<210> 17

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Description of artificial sequence: Primer for the
detection of human acid ceramidase mutation A107G

<400> 17

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20

<210> 18

<211> 33

<212> DNA

<213> Artificial sequence

<220>

<223> Description of artificial sequence: Template of
human acid ceramidase; r = a, wild type template;
r = g, mutant template

<400> 18

aatcaacct rtctctcttc aggaccaacg tac

33

<210> 19

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Description of artificial sequence: Primer probe
BrafT

<400> 19

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22

<210> 20

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Description of artificial sequence: Reverse
primer for BRAF

<400> 20

agaggaaaga tgaagtacta tg

22

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<210> 21
<211> 239
<212> DNA
<213> Artificial sequence

<220>

<223> Description of artificial sequence: Target template
BrafX; w = a, Braf A (wild type); w = t, BrafT (mutant)

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<210> 22
<211> 25
<212> DNA
<213> Artificial sequence

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<223> Description of artificial sequence: Primer probe
DpyDT

<400> 22
gttttagatg ttaaatacaca cttat 25

<210> 23
<211> 23
<212> DNA
<213> Artificial sequence

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<223> Description of artificial sequence: Reverse
primer for DpyDT

<400> 23
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<210> 24
<211> 300
<212> DNA
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<223> Description of artificial sequence: Target template
DpyDX; r = a, DpyDA (wild type); r = t, DpyDT (mutant)

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<210> 25
 <211> 7043
 <212> DNA
 <213> Artificial sequence

<220>

<223> Description of artificial sequence: pTTQ18::Taq

<400> 25

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<223> Description of artificial sequence: pQE30

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